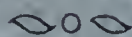


THE CONSTITUENTS
OF
WITHANIA SOMNIFERA

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LIX.—*The Constituents of Withania somnifera.*

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THE plant *Withania somnifera*, Dunal (Nat. ord. *Solanaceae*), occurs in South Africa, where it is found in gardens and waste places, and is known under the Kaffir name of *ubu-Vumba*. It is also a native of the west coast of India, and is said to be common along the shores of the Mediterranean. The plant is described in De Candolle's *Prodromus*, Vol. 13, Part I, p. 453, and has been recorded by

J. Medley Wood in a List of the Flora of Natal (*Trans. South African Phil. Soc.*, 1908, **18**, Part II, 197), while a more extended description of its characters and uses is given in Smith's "Contribution to South African Materia Medica," pp. 145, 166, and in the "Pharmacographia Indica," Vol. II, p. 566. Although various medicinal properties have been attributed to the plant, it is particularly stated to act as a sedative and hypnotic (*Amer. J. Pharm.*, 1891, **63**, 77), and this action has been considered by Trebut (*The Lancet*, 1886, **1**, 467) to be due to the presence of an alkaloid, which was provisionally designated by him "somniferine." Inasmuch as nothing of a definite nature has been recorded respecting this alkaloid or the other constituents of the plant, it seemed desirable to subject it to a more complete examination, and the results are summarised at the end of this paper.

EXPERIMENTAL.

The material used for the present investigation was received directly from South Africa, and consisted of both the root and the overground portion of the plant.

I. *Examination of the Root.*

A small portion of the root was tested for the presence of an alkaloid, with a positive result.

Fifty grams of the ground material were subsequently extracted in a Soxhlet apparatus with various solvents, when the following amounts of extract, dried at 100°, were obtained:

Petroleum (b. p. 35—40°)	extracted	0·22 gram	=0·44	per cent.
Ether	"	0·35 "	=0·70	" "
Chloroform	"	0·23 "	=0·46	" "
Ethyl acetate	"	0·35 "	=0·70	" "
Alcohol	"	2·15 "	=4·30	" "

Total..... 3·30 grams or 6·60 per cent.

For the purpose of a complete examination, 18·37 kilograms of the ground root were extracted by continuous percolation with hot alcohol. After the removal of the greater portion of the alcohol, there remained a dark-coloured, viscid extract, which amounted to 2·7 kilograms.

Distillation of the Extract with Steam: Separation of an Essential Oil.

The whole of the above extract was mixed with water, and the mixture distilled in a current of steam for several hours. The distillate, which contained a small amount of an essential oil, was

shaken with ether, the ethereal liquid being washed, dried, and the solvent removed. The essential oil thus obtained possessed a light brown colour, a pungent odour, and amounted to 1·2 grams, being thus equivalent to about 0·006 per cent. of the weight of the root.

Non-volatile Constituents of the Extract.

After the above-described operation, there remained in the distillation flask a dark-coloured, aqueous liquid (A), and a considerable quantity of a black resin (B). These products were separated by filtration, and the resin thoroughly washed with water, the washings being added to the main portion of the aqueous liquid.

Examination of the Aqueous Liquid (A).

The aqueous liquid was concentrated under diminished pressure to a convenient volume, and then repeatedly extracted with ether. A small amount (4·5 grams) of a viscid, brown oil was thus removed, which, however, with the exception of traces of a weak base, yielded nothing of interest.

The aqueous liquid was next shaken repeatedly with amyl alcohol. This solvent extracted a considerable quantity of a yellow, amorphous product, which, on exposure to the atmosphere, rapidly absorbed moisture, and became glutinous. This yellow solid could not be obtained crystalline, nor could any crystalline derivative be prepared from it. It was not glucosidic.

The aqueous liquid was subsequently treated with a slight excess of basic lead acetate, when a voluminous, light brown precipitate was obtained. The lead precipitate, which contained nothing of an alkaloidal nature, was examined in the usual way, but yielded only indefinite substances, giving the reactions for tannin. The filtrate from the basic lead acetate precipitate was treated with hydrogen sulphide for the removal of the lead, and the mixture filtered. The concentrated filtrate was yellow in colour, and contained a considerable quantity of sugar, since it readily yielded *d*-phenylglucosazone, melting and decomposing at 210°. On heating the liquid with alkali hydroxide, it developed ammonia, and it also yielded precipitates with the usual alkaloid reagents, but these reactions were evidently due to soluble protein products, since the alkaline liquid, when extracted with either chloroform or amyl alcohol, yielded nothing of a definitely alkaloidal nature.

Examination of the Resin (B).

The resin amounted to 502 grams, being thus equivalent to about 2·7 per cent. of the weight of the root. It was digested with hot

alcohol, the solution poured upon purified sawdust, and the dried mixture extracted successively in a Soxhlet apparatus with light petroleum, ether, chloroform, ethyl acetate, and alcohol.

Petroleum Extract of the Resin.

This was a dark-coloured, soft solid, which amounted to 138 grams. It was heated in a reflux apparatus with an excess of an alcoholic solution of potassium hydroxide, the greater portion of the alcohol then removed, water added, and the alkaline mixture extracted with ether. On evaporating the solvent, a quantity (37 grams) of a brown, viscid product was obtained, which was found to contain some basic substance. It was therefore re-dissolved in ether, and the ethereal solution shaken with dilute hydrochloric acid. The acid liquid was then carefully neutralised with sodium carbonate, when a colourless precipitate, amounting to about 0.1 gram, was deposited. This substance, when recrystallised from dilute alcohol, separated in colourless, glistening leaflets, melting at 116° , and was found to be identical with the base, $C_{12}H_{16}N_2$, subsequently to be described in connexion with the examination of the ether extract of the resin. As will be shown later, it is probable that the above compound is not originally present in the resin, but was formed from an alkaloidal constituent by the treatment with alcoholic potassium hydroxide.

Isolation of Hentriacontane, $C_{31}H_{64}$, and a Phytosterol, $C_{27}H_{46}O$.

The ethereal solution, which had been shaken with hydrochloric acid for the removal of the base, as described above, was washed, dried, and the solvent evaporated. The residue was then fractionally crystallised from a mixture of ethyl acetate and alcohol. The more sparingly soluble deposits, when crystallised from ethyl acetate, were obtained in glistening leaflets, melting at $67-68^{\circ}$, and proved to be hentriacontane. (Found, $C=85.2$; $H=14.5$. Calc., $C=85.3$; $H=14.7$ per cent.)

After the removal of the greater part of the hentriacontane in the above fractionation, the mother liquors gradually deposited a small quantity of a substance in flat plates. This was purified by recrystallisation from ethyl acetate, and it then melted at $135-136^{\circ}$:

0.1113 * gave 0.3420 CO_2 and 0.1202 H_2O . $C=83.8$; $H=12.0$.

$C_{27}H_{46}O$ requires $C=83.9$; $H=11.9$ per cent.

This substance was evidently a phytosterol, and it gave the colour reaction of that class of compounds.

* Anhydrous substance.

*The Fatty Acids.**Isolation of Cerotic Acid, $C_{26}H_{52}O_2$.*

The alkaline, aqueous solution of potassium salts, which had been extracted with ether for the removal of the unsaponifiable material, as above described, was acidified with sulphuric acid, and extracted with ether. On evaporating the ethereal solution, it yielded 15 grams of a dark green, soft solid, which evidently contained a considerable amount of resinous matter. The latter was separated by washing with petroleum, in which the resin was insoluble. The mixed fatty acids obtained from the petroleum washings were converted into their methyl esters, and the latter distilled under diminished pressure. Two fractions were collected, the one distilling at $205\text{--}230^\circ/28\text{ mm.}$, and the other above $230^\circ/28\text{ mm.}$ The latter fraction, consisting of a wax-like solid, yielded, on hydrolysis, an acid which, on recrystallisation from alcohol, separated in clusters of colourless leaflets, melting at $71\text{--}72^\circ$, and was identified as cerotic acid (Found, $C=78.7$; $H=13.2$. Calc., $C=78.8$; $H=13.1$ per cent.), the somewhat low melting point being probably due to a slight impurity.

The above-mentioned fraction, which distilled at $205\text{--}230^\circ/28\text{ mm.}$, contained a considerable proportion of unsaturated esters. The entire fraction was therefore hydrolysed, and the resulting fatty acids separated by means of their lead salts into saturated and unsaturated portions. The saturated acids, amounting to 5.5 grams, were crystallised from alcohol, and thus separated into two fractions of different solubilities. Each of these fractions melted at $52\text{--}54^\circ$, but they possessed neutralisation values of 210 and 201 respectively, and it was thus evident that the saturated acids consisted of a mixture of palmitic and stearic acids.

The unsaturated acids, which amounted to 4.8 grams, were distilled under diminished pressure, when they passed over at $220\text{--}240^\circ/20\text{ mm.}$ An analysis and a determination of the iodine value gave the following results:

0.1391 gave 0.3916 CO_2 and 0.1462 H_2O . $C=76.8$; $H=11.7$.

0.1711 absorbed 0.2260 iodine. Iodine value = 132.1.

$C_{18}H_{34}O_2$ requires $C=76.6$; $H=12.1$ per cent. Iodine value = 90.1.

$C_{18}H_{32}O_2$,, $C=77.1$; $H=11.4$,, Iodine value = 181.4.

These results indicate that the unsaturated acids consisted of a mixture of oleic and linolic acids in approximately equal proportions.

*Ethereal Extract of the Resin.**Isolation of Ipuranol, $C_{23}H_{38}O_2(OH)_2$.*

The ether extract of the resin was a black, brittle solid, amounting to 42.7 grams. It was digested with a large volume of ether, when about 4 grams of a light green, solid substance remained undissolved. The latter was collected, and purified by several crystallisations from pyridine containing a little water, when it was obtained in colourless, microscopic needles, melting and decomposing at 290—300°:

0.1103 gave 0.2929 CO_2 and 0.1021 H_2O . $C = 72.4$; $H = 10.3$.

$C_{23}H_{40}O_4$ requires $C = 72.6$; $H = 10.5$ per cent.

When the substance was dissolved in chloroform with a little acetic anhydride, and a drop of concentrated sulphuric acid subsequently added, a transient pink colour, changing to blue and then to green, was produced. The compound also yielded an acetyl derivative, melting at 164—165°, and it was thus definitely identified as ipuranol.

The ethereal solution from which the sparingly soluble ipuranol had been removed, as above described, was extracted successively with aqueous ammonium carbonate, sodium carbonate, and sodium hydroxide. These extracts, however, on acidification, yielded only resinous material. On evaporating the greater portion of the ether, a small quantity of a crystalline compound separated, which, when purified by crystallisation from a mixture of alcohol and chloroform, separated in thin, colourless needles, melting and decomposing at 300°. It was found to be identical with the new compound, withaniol, $C_{25}H_{34}O_5$, which was obtained in larger amount from the chloroform extract of the resin.

Isolation of an Alkaloidal Principle.

The ethereal filtrate from the above-described crystalline substance contained a compound which gave precipitates with the usual alkaloid reagents. The alkaloidal principle was not soluble in dilute acids, but was extracted from the ethereal liquid by repeatedly shaking with concentrated hydrochloric acid. The acid liquids were then carefully neutralised with sodium carbonate, when an amorphous solid was precipitated, which was taken up with chloroform. The chloroform extract, after washing and drying, yielded 1.5 grams of a weak, gum-like base, which could not be obtained in a crystalline state. It gave a yellow, amorphous gold salt, melting and decomposing at 185°.

Action of Potassium Hydroxide on the Alkaloid. Isolation of a Crystalline Base, $C_{12}H_{16}N_2$.

A portion of the above-described alkaloid was heated for an hour with an alcoholic solution of potassium hydroxide. The alcohol was then removed, water added, and the alkaline liquid extracted with ether. The ethereal solution was then shaken with dilute hydrochloric acid, and the acid liquid neutralised with aqueous sodium carbonate, when a small amount (0.2 gram) of a colourless solid was precipitated. This substance was crystallised from dilute alcohol, and was thus obtained in colourless, glistening leaflets, melting at 116° :

0.0792 gave 0.2230 CO_2 and 0.0597 H_2O . $C=76.8$; $H=8.4$.

0.0655 „ 8.5 c.c. moist N_2 (over KOH) at 14° and 760 mm.
 $N=15.3$.

$C_{12}H_{16}N_2$ requires $C=76.6$; $H=8.5$; $N=14.9$ per cent.

The above-described substance would thus appear to possess the empirical formula $C_{12}H_{16}N_2$, and, as it differs in its properties from any substance of this formula which has hitherto been recorded, it is evidently a new compound.

The *base*, $C_{12}H_{16}N_2$, is readily soluble in alcohol, ethyl acetate, ether, chloroform, or benzene, but only sparingly soluble in light petroleum or hot water. It is neutral towards litmus, but is easily dissolved by an excess of dilute mineral acids. When heated in an ignition tube, it first melts, and then sublimes unchanged.

The *hydrochloride* was prepared by adding to an ethereal solution of the base a drop of concentrated hydrochloric acid. A crystalline solid was thus deposited, which, when recrystallised from a mixture of ether and alcohol, separated in thin, colourless needles, sintering at 190° , and melting at 201° . The *picrate* crystallised from hot water, in which it is only moderately soluble, in silky, yellow needles, melting at 171° .

After the removal of the crystalline base from the product of the above reaction, the alkaline liquid yielded, on acidification, a small quantity of a solid substance, which, however, could not be obtained crystalline.

Chloroform Extract of the Resin.

Isolation of a New Monohydric Alcohol, Withaniol, $C_{25}H_{33}O_4 \cdot OH$.

The portion of the resin extracted by chloroform was relatively large, amounting to 123 grams. It was redissolved in chloroform, and the solution extracted with aqueous ammonium carbonate, sodium carbonate, and sodium hydroxide, each of which removed

some amorphous, resinous material. The chloroform solution was then washed, dried, and the solvent removed, when a viscid, brown residue was obtained, which, on agitation with alcohol, deposited a colourless, crystalline compound. This compound, amounting to 5 grams, was collected, and recrystallised from a mixture of chloroform and alcohol, when it separated in colourless needles, which sintered at 285° and completely decomposed at 305° :

0.1081 gave 0.2866 CO_2 and 0.0816 H_2O . $\text{C}=72.2$; $\text{H}=8.4$.

0.1022 „ 0.2703 CO_2 „ 0.0750 H_2O . $\text{C}=72.1$; $\text{H}=8.2$.

The molecular weight of the substance was determined by the cryoscopic method:

0.3170, in 28.16 nitrobenzene, gave $\Delta t = -0.191^{\circ}$. M.W.=412.

$\text{C}_{25}\text{H}_{34}\text{O}_5$ requires $\text{C}=72.5$; $\text{H}=8.2$ per cent. M.W.=414.

No compound of the empirical formula $\text{C}_{25}\text{H}_{34}\text{O}_5$, possessing properties identical with those of the above substance, has hitherto been described. It is therefore proposed to designate the new compound *withaniol*, with reference to the generic name of the plant from which it has been obtained.

Withaniol, $\text{C}_{25}\text{H}_{34}\text{O}_5$, is readily soluble in chloroform, only moderately so in alcohol, and practically insoluble in ether or light petroleum. It dissolves in cold concentrated hydrochloric acid, and is precipitated unchanged on the addition of water. It undergoes some change when heated with alkalis in the presence of alcohol, since the solution becomes deep red, and deposits, on acidification, a red, amorphous precipitate. When dissolved in a mixture of acetic anhydride and chloroform, and a drop of concentrated sulphuric acid subsequently added, a pink coloration appears, which gradually changes to a straw-yellow colour.

Withaniol is optically active, a determination of its rotatory power giving the following result:

0.3745, made up to 20 c.c. with chloroform, gave $\alpha_D + 3^{\circ}15'$ in a 2-dcm. tube, whence $[\alpha]_D + 91.2^{\circ}$.

Withaniol contains no methoxy-group, but the presence of one hydroxyl group was proved by the formation of a monoacetyl derivative.

Acetylwithaniol, $\text{C}_{25}\text{H}_{33}\text{O}_5 \cdot \text{CO} \cdot \text{CH}_3$.—This derivative was prepared by heating *withaniol* for some time with an excess of acetic anhydride. On cooling the solution, the *acetyl* compound was deposited in the form of colourless, prismatic needles. After recrystallisation from acetic anhydride, it began to melt at 280° , and at 300° became completely decomposed:

0.1032 gave 0.2675 CO_2 and 0.0750 H_2O . $\text{C}=70.7$; $\text{H}=8.1$.

0.1122 „ 0.2920 CO_2 „ 0.0832 H_2O . $\text{C}=71.0$; $\text{H}=8.2$.

$\text{C}_{25}\text{H}_{33}\text{O}_5 \cdot \text{CO} \cdot \text{CH}_3$ requires $\text{C}=71.1$; $\text{H}=7.9$ per cent

Ethyl Acetate Extract of the Resin.

This was a black, brittle solid, amounting to 26.8 grams. It was heated for several hours with dilute sulphuric acid in aqueous alcohol, when, after removing the alcohol, an amorphous resin separated, and a yellow, aqueous liquid was obtained which readily reduced Fehling's solution. The presence of sugar in the aqueous liquid was confirmed by the formation of *d*-phenylglucosazone, melting and decomposing at 210°. It is thus evident that the ethyl acetate extract of the resin was glucosidic in character.

Alcohol Extract of the Resin.

This resin amounted to 105 grams, and consisted of a hard, black solid. A preliminary examination having indicated the presence of a glucosidic substance, the whole of the resin was heated for several hours with dilute sulphuric acid in aqueous alcohol. After the removal of the alcohol, the mixture was distilled in a current of steam, but no volatile product of hydrolysis was obtained. There then remained in the distillation flask a quantity of resin and a reddish-brown, aqueous liquid, which was separated by filtration. The resin yielded nothing crystalline, but the aqueous liquid, when rendered alkaline by means of sodium hydroxide, deposited about 1 gram of a dark brown, gelatinous product. This was collected, found to contain nitrogen, and to possess weakly basic properties. It was, however, of a very indefinite character, and could not be obtained crystalline, nor could any crystalline salt be prepared from it. When heated with aqueous potassium hydroxide, a strong ammoniacal odour was developed.

The aqueous liquid, from which the above-described basic substance had been removed, readily reduced Fehling's solution, and yielded *d*-phenylglucosazone, melting and decomposing at 205°. It is thus apparent that at least a portion of the alcohol extract of the resin was of a glucosidic nature.

II. Examination of the Leaves and Stems.

As a preliminary experiment, a small portion of the ground material was tested for the presence of an alkaloid, and with a positive result.

For the purpose of a complete examination, 6.97 kilograms of the dried leaves and stems were ground, and extracted by continuous percolation with hot alcohol. The greater part of the alcohol was then removed, the residual extract (2.24 kilograms) mixed with water, and the mixture distilled in a current of steam. The

distillate contained a very small quantity (0.2 gram) of an essential oil, possessing a strong, pungent odour.

After the above-described operation, there remained in the distillation flask a brown, aqueous liquid, containing a quantity of resin. These products were separated by decantation, and the resin well washed with boiling water, the washings being added to the main volume of aqueous liquid.

Examination of the Aqueous Liquid.

The aqueous liquid was concentrated under diminished pressure to a convenient bulk, then repeatedly extracted with ether, and finally with amyl alcohol. These solvents, however, removed only small quantities of gummy material, which, in each case, was found to contain a trace of a weak amorphous base. The latter, although responding to the usual tests for an alkaloid, was very indefinite in character, and yielded no crystalline salt. No glucosidic substance was present.

The aqueous liquid which had been extracted with the above-mentioned solvents was subsequently treated with an excess of basic lead acetate, which removed tannin and colouring matter. The filtrate from the basic lead acetate precipitate was deprived of lead and concentrated under diminished pressure to a small volume, when, on cooling, a considerable quantity of potassium nitrate was deposited. The filtrate from the latter still gave a precipitate with alkaloid reagents, and also contained some sugar, since it readily yielded *d*-phenylglucosazone, decomposing at 205°. The aqueous liquid was finally rendered alkaline and extracted successively with ether and chloroform, but these solvents removed only very small quantities of a gummy, basic substance, which yielded no crystalline derivative.

Examination of the Resin.

The resin was a dark green powder, and amounted to 624 grams. It was dissolved in hot alcohol, mixed with purified sawdust, and the thoroughly dried mixture extracted successively in a Soxhlet apparatus with light petroleum, ether, chloroform, ethyl acetate, and alcohol.

Petroleum Extract of the Resin.

This extract was a dark green, soft solid, amounting to 67 grams. It was dissolved in ether, and the solution shaken with both dilute and strong hydrochloric acid, but no basic substance was thus removed.

Isolation of Hentriacontane, C₃₁H₆₄, and a Phytosterol, C₂₇H₄₆O.

The above-mentioned ethereal liquid was next distilled for the removal of the solvent, and the residue heated with an alcoholic solution of potassium hydroxide. After the removal of the alcohol, water was added, and the alkaline liquid completely extracted with ether. The ethereal extract yielded 26 grams of a semi-solid product, which was fractionally crystallised from ethyl acetate, when a colourless compound, separating in glistening leaflets and melting at 68°, was obtained. This substance, amounting to 5 grams, was identified as hentriacontane (Found, C=85·1; H=14·7. Calc., C=85·3; H=14·7 per cent.).

The mother-liquors from the above crystallisations were united, the solvent removed, and the residue fractionally distilled under diminished pressure. The lower-boiling fractions contained a considerable quantity of hentriacontane, but the final fraction, after several crystallisations from hot alcohol, yielded a compound crystallising in flat plates, which melted at 133°, and gave the colour reaction of the phytosterols:

0·0883 gave 0·2720 CO₂ and 0·0940 H₂O. C=84·0; H=11·8.

C₂₇H₄₆O requires C=83·9; H=11·9 per cent.

This substance was thus proved to be a phytosterol, and it was apparently identical with that obtained from the root.

*The Fatty Acids.**Isolation of Cerotic Acid, C₂₆H₅₂O₂.*

The alkaline liquid from which the above-mentioned hentriacontane and phytosterol had been removed was acidified with sulphuric acid, and the precipitated fatty acids taken up with ether. The ethereal solution, on the evaporation of the solvent, yielded a semi-solid residue of fatty acids, which were converted into their methyl esters, and the latter then fractionally distilled under diminished pressure. The greater portion passed over at 205—215°/12 mm., but a small fraction was collected above 215°/12 mm., and a considerable quantity of non-volatile resinous material remained in the distillation flask. The fraction distilling above 215°/12 mm. solidified in the receiver, and, when recrystallised from alcohol, was obtained in glistening leaflets, melting at 56—57°. This product consisted of methyl cerotate (m. p. 60°), since on hydrolysis it yielded an acid which separated from ethyl acetate in stellar aggregates of fine needles, melting at 76—77° (Found, C=78·6; H=13·3. Calc., C=78·8; H=13·1 per cent.).

The fraction of methyl esters distilling at 205—215°/12 mm. was hydrolysed, and the regenerated acids separated by means of their lead salts into saturated and unsaturated portions. The saturated acids were found to melt at 49—51°, and to possess a neutralisation value of 205°. After one crystallisation from alcohol, the melting point was 51—53° and the neutralisation value 207°. It was thus evident that the saturated acids consisted of a mixture of palmitic and stearic acids, which possess neutralisation values of 219 and 198 respectively.

The unsaturated acids were distilled under diminished pressure, when they passed over at 220—235°/15 mm. An analysis and a determination of the iodine value gave the following results:

0.1033 gave 0.2912 CO₂ and 0.1075 H₂O. C=76.9; H=11.6.

0.1306 absorbed 0.2086 iodine. Iodine value=159.7.

C₁₈H₃₄O₂ requires C=76.6; H=12.1 per cent. Iodine value=90.1.

C₁₈H₃₂O₂ „ C=77.1; H=11.4 „ „ Iodine value=181.4.

The unsaturated acids would thus appear to have consisted of a mixture of oleic and linolic acids, the latter preponderating.

Ethereal Extract of the Resin.

Isolation of Ipuranol, C₂₃H₃₈O₂(OH)₂.

This extract was obtained in the form of a light green powder, amounting to 156 grams. On digesting the extract with ether, it was observed that a portion of it was very sparingly soluble. This was therefore separated by filtration from the more readily soluble portion, which amounted to 60 grams, and consisted of chlorophyll and indefinite amorphous substances. The portion sparingly soluble in ether was subsequently digested with hot alcohol, when a small quantity of a light green solid remained undissolved. The latter was collected, and, after crystallisation from dilute pyridine, was found to decompose at 285—290°, and to give the colour reaction of ipuranol. Its complete identification was effected by means of the acetyl derivative, which melted at 164—165°, and when mixed with a known specimen of acetylipuranol the melting point remained unchanged.

Isolation of a New Monohydric Alcohol, Somnirol, C₃₂H₄₈O₆·OH.

The above-mentioned alcoholic solution of the sparingly soluble portion of the ether extract of the resin was heated to boiling, and sufficient water added to produce a faint cloudiness. On cooling, a quantity of an oily resin separated, which gradually became semi-solid. This was collected and put aside for subsequent examination. By the further cautious addition of water

to the hot mother-liquors, the greater portion of the oleo-resinous material was removed, after which a crystalline substance began to be deposited. The latter was then purified by several recrystallisations from dilute alcohol, when it was obtained in colourless, prismatic needles in an amount of 3 grams:

0.5981, dried at 110° , lost 0.0364 H_2O . $\text{H}_2\text{O}=6.1$.

0.0975* gave 0.2542 CO_2 and 0.0748 H_2O . $\text{C}=71.1$; $\text{H}=8.5$.

0.1126* „ 0.2882 CO_2 „ 0.0834 H_2O . $\text{C}=71.0$; $\text{H}=8.4$.

0.1136* „ 0.2968 CO_2 „ 0.0844 H_2O . $\text{C}=71.3$; $\text{H}=8.2$.

$\text{C}_{32}\text{H}_{44}\text{O}_7 \cdot 2\text{H}_2\text{O}$ requires $\text{H}_2\text{O}=6.2$ per cent.

$\text{C}_{32}\text{H}_{44}\text{O}_7$ requires $\text{C}=71.1$; $\text{H}=8.1$ per cent.

The molecular weight of the substance was determined by the cryoscopic method with the following result:

0.5496*, in 31.52 nitrobenzene, gave $\Delta t = -0.225^{\circ}$. M.W.=542.

$\text{C}_{32}\text{H}_{44}\text{O}_7$ requires M.W.=540.

No substance identical with the above compound appears to have hitherto been described. It is therefore proposed to designate the new compound *somnirol*, with reference to its alcoholic properties and the specific name of the plant from which it has been isolated.

Somnirol, $\text{C}_{32}\text{H}_{43}\text{O}_6 \cdot \text{OH}$, is very soluble in alcohol, ethyl acetate, or chloroform, only moderately soluble in benzene, and very sparingly so in ether. It crystallises from dilute alcohol with two molecules of water of crystallisation, which are expelled at 110° , but are again rapidly absorbed when the anhydrous substance is exposed to the atmosphere. The behaviour of the substance on heating is characteristic, inasmuch as it partly melts at about 155° with effervescence and immediate resolidification, and then finally fuses with evolution of gas at 205° .

A determination of the optical rotatory power of somnirol gave the following result:

0.1796,* made up to 25 c.c. with chloroform, gave $\alpha_D + 0^{\circ}30'$ in a 2-dcm. tube, whence $[\alpha]_D + 34.8^{\circ}$.

Somnirol contains no methoxyl, and, as shown below, only one hydroxyl group. In order to obtain some further information respecting the character of the substance, a portion of it was heated with decinormal alcoholic potassium hydroxide:

0.2002, heated for 20 minutes, neutralised 0.0222 KOH.

KOH=11.1 per cent.

0.2271, heated for one hour, neutralised 0.0275 KOH.

KOH=12.1 per cent.

$\text{C}_{32}\text{H}_{44}\text{O}_7$, to neutralise 1 mol. KOH, requires KOH=10.4 per cent.

With the object of isolating, if possible, the products of the

* Anhydrous substance.

above reaction, a quantity (0.5 gram) of somnirol was heated for a short time with alcoholic potassium hydroxide. The alcohol was then removed and water added, but no precipitation ensued, nor could any product be isolated from the alkaline solution by extraction with ether or chloroform. The alkaline liquid, on acidification, deposited a gelatinous precipitate, which, after collecting and drying, was found to be only imperfectly soluble in alkalis, and almost insoluble in the ordinary organic solvents. From these results it may be concluded that somnirol contains at least one lactonic grouping.

Acetylsomnirol, $C_{32}H_{43}O_7 \cdot CO \cdot CH_3$.—This compound was prepared by heating somnirol for an hour with acetic anhydride, the greater portion of the latter being then removed by distillation, and water added, when the *acetyl* derivative was precipitated as an oil, which gradually became solid. The product was collected, dried in a vacuum over sulphuric acid, and dissolved in hot benzene, from which it was deposited in colourless, torpedo-shaped crystals. The latter contained benzene of crystallisation, which was not lost on keeping the compound for some time in a vacuum over sulphuric acid, and was only very slowly expelled on heating at 100° . When heated in a capillary tube, the air-dried substance begins to change at about 115° , and apparently melts at 125° with evolution of gas. Heated to 110° on a watch-glass, the substance gradually melts, bubbles of gaseous benzene are evolved, and it then resolidifies to a hard, brittle, transparent mass. On recrystallising the latter from benzene, the original compound, containing that solvent, was obtained:

0.1178 gave 0.3157 CO_2 and 0.0859 H_2O . $C=73.1$; $H=8.1$.

$C_{32}H_{43}O_7 \cdot CO \cdot CH_3, C_6H_6$ requires $C=72.7$; $H=7.9$ per cent.

The benzene-free acetyl derivative was next analysed:

0.1369 gave 0.3510 CO_2 and 0.0994 H_2O . $C=69.8$; $H=8.1$.

0.0801 „ 0.2050 CO_2 „ 0.0586 H_2O . $C=69.8$; $H=8.1$.

$C_{32}H_{43}O_7 \cdot CO \cdot CH_3$ requires $C=70.1$; $H=7.9$ per cent.

Isolation of a New Dihydric Alcohol, Somnitol, $C_{33}H_{44}O_5(OH)_2$.

The dilute alcoholic mother-liquors remaining after the separation of the somnirol, as above described, still contained a considerable amount of substance in solution. On removing the alcohol in a current of steam, an oily substance was deposited, which gradually solidified. The solid was collected, dried in a vacuum over sulphuric acid, and then digested with ethyl acetate. This treatment removed the remaining somnirol, and left a sparingly soluble compound, which was recrystallised from alcohol. It separated from

this solvent in thin, hair-like needles, which sintered at 240° , and melted and decomposed at about 250° . The yield of this substance was 4 grams:

0.4704, dried at 110° , lost 0.0310 H_2O . $\text{H}_2\text{O}=6.6$.

0.1160* gave 0.3037 CO_2 and 0.0898 H_2O . $\text{C}=71.4$; $\text{H}=8.6$.

0.1071* „ 0.2805 CO_2 „ 0.0830 H_2O . $\text{C}=71.4$; $\text{H}=8.6$.

$\text{C}_{33}\text{H}_{46}\text{O}_7 \cdot 2\text{H}_2\text{O}$ requires $\text{H}_2\text{O}=6.1$ per cent.

$\text{C}_{33}\text{H}_{46}\text{O}_7$ requires $\text{C}=71.5$; $\text{H}=8.3$ per cent.

A molecular-weight determination by the cryoscopic method gave the following result:

0.2454,* in 33.80 nitrobenzene, gave $\Delta t = -0.092^{\circ}$. $\text{M.W.}=553$.

$\text{C}_{33}\text{H}_{46}\text{O}_7$ requires $\text{M.W.}=554$.

No compound of the above formula appears to have previously been described. It is therefore proposed to designate the substance *somnitol*, with reference to its alcoholic character and the specific name of the plant from which it has been obtained.

Somnitol is practically insoluble in water, and only moderately soluble in hot absolute alcohol. It dissolves readily in chloroform and in dilute alcohol, separating from the latter solvent with 2 molecules of water. The compound is optically active, a determination of its rotatory power giving the following result:

0.3040,* made up to 25 c.c. with chloroform, gave $\alpha_D + 0^{\circ}31'$ in a 2-dcm. tube, whence $[\alpha]_D + 21.2^{\circ}$.

When *somnitol* is heated with alkali hydroxides in alcoholic solution it slowly undergoes change, and the liquid acquires a deep red colour, but even after prolonged heating with concentrated alkali a part of the compound was recovered unaltered. The reaction is therefore not comparable with that which takes place when *somnirol* is similarly treated, since in the latter case no trace of unchanged substance can be detected, even after a few minutes' heating with the alkali.

Somnitol contains no methoxyl, but was found to possess two hydroxyl groups, since it readily yields a diacetyl derivative.

Diacetylsomnitol, $\text{C}_{33}\text{H}_{44}\text{O}_7(\text{CO} \cdot \text{CH}_3)_2$.—This compound was prepared by heating *somnitol* with acetic anhydride for an hour. On concentrating the solution, an *acetyl* derivative separated in colourless prisms, which, after recrystallisation from acetic anhydride, melted and decomposed at 270 — 275° . It was practically insoluble in cold acetic anhydride or alcohol, but readily soluble in chloroform:

0.1127 gave 0.2866 CO_2 and 0.0841 H_2O . $\text{C}=69.4$; $\text{H}=8.3$.

0.1056 „ 0.2693 CO_2 „ 0.0782 H_2O . $\text{C}=69.6$; $\text{H}=8.2$.

$\text{C}_{33}\text{H}_{44}\text{O}_7(\text{CO} \cdot \text{CH}_3)_2$ requires $\text{C}=69.6$; $\text{H}=7.8$ per cent.

* Anhydrous substance.

Isolation of an Acidic, Hydrolytic Product: Withanic Acid,
 $C_{29}H_{45}O_6 \cdot CO_2H$.

The oily resin which had separated from the alcoholic solution of the ether extract of the resin by the first addition of water, as above described, was next examined, but no crystalline compound could be directly isolated from it. It was therefore heated for a short time with an alcoholic solution of potassium hydroxide, the alcohol then removed, and the product of hydrolysis extracted successively with ether and chloroform, which, however, removed nothing. On acidifying the alkaline liquid, a gelatinous precipitate was formed, the greater part of which was insoluble in organic solvents, but a small portion was soluble in hot alcohol, from which it crystallised in colourless, tetrahedral prisms, melting and decomposing at 226° :

0.1124, dried at 110° , lost 0.0038 H_2O . $H_2O = 3.1$.

0.1136* gave 0.2796 CO_2 and 0.0904 H_2O . $C = 67.1$; $H = 8.8$.

$C_{30}H_{46}O_8 \cdot H_2O$ requires $H_2O = 3.3$ per cent.

$C_{30}H_{46}O_8$ requires $C = 67.4$; $H = 8.6$ per cent.

This substance was readily soluble in alkalis, including ammonium carbonate, and when titrated with a decinormal alcoholic solution of sodium hydroxide gave the following result:

0.2060 neutralised 3.8 c.c. $N/10$ - $NaOH$. M.W. (monobasic acid) = 542.

$C_{29}H_{45}O_6 \cdot CO_2H$ requires M.W. = 534.

As the above-described acid is evidently a new compound, it is proposed to designate it *withanic acid*.

Withanic acid, $C_{30}H_{46}O_8$, is very sparingly soluble in ether, chloroform, or benzene, and only moderately soluble in boiling alcohol. Its *methyl* ester, prepared by heating the substance with methyl alcohol in presence of concentrated sulphuric acid, was very soluble in alcohol or acetone, but could be crystallised from a mixture of acetone and benzene, when it separated in hard, crystalline nodules, sintering at 240° , and decomposing with effervescence at 255° .

Chloroform Extract of the Resin.

This consisted of a light green powder, which amounted to 212 grams. As nothing could be directly isolated from this material, it was heated with dilute sulphuric acid in aqueous alcohol. On subsequently rendering the liquid alkaline, about 1 gram of an almost colourless, glutinous solid separated, which

* Anhydrous substance.

was found to contain nitrogen, but which could not be obtained crystalline nor did it yield any crystalline salt.

The chloroform extract of the resin was also tested for the presence of a glucoside, but with a negative result.

Ethyl Acetate and Alcohol Extracts of the Resin.

The ethyl acetate extract of the resin amounted to only 10 grams. It was found to contain some glucosidic material, since on heating with dilute sulphuric acid in aqueous alcohol it yielded a sugar from which *d*-phenylglucosazone (m. p. 205°) was prepared.

The alcohol extract of the resin, consisting of a hard, brittle solid, amounted to 94 grams. It was glucosidic in character, and on heating for several hours with dilute sulphuric acid in aqueous alcohol it also yielded a small amount of an indefinite basic substance, the properties of which were very similar to those of the substance obtained from the chloroform extract of the resin.

Summary.

The material employed for this investigation represented the entire plant of *Withania somnifera*, Dunal (Nat. ord. *Solanaceae*), and was obtained directly from South Africa. The root and the overground portion of the plant, consisting of the leaves and stems, were separately examined.

Preliminary tests, conducted with both portions of the plant, indicated the presence of an alkaloid.

I. *Constituents of the Root.*—An alcoholic extract of the root, when distilled with steam, yielded a very small amount of an essential oil. The portion of the extract which was soluble in water contained, besides indefinite, amorphous substances, a quantity of sugar, which yielded *d*-phenylglucosazone (m. p. 210°).

The portion of the extract which was insoluble in water consisted chiefly of a black resin, and amounted to about 2·7 per cent. of the weight of the root. From this resin the following definite substances were isolated: hentriacontane, $C_{31}H_{64}$; a phytosterol, $C_{27}H_{46}O$ (m. p. 135—136°); a mixture of fatty acids, consisting of palmitic, stearic, cerotic, oleic, and linolic acids; ipuranol, $C_{23}H_{38}O_2(OH)_2$; a new monohydric alcohol, *withaniol*, $C_{25}H_{33}O_4 \cdot OH$, decomposing at 305°, and having $[\alpha]_D + 91\cdot2^\circ$; and an amorphous, alkaloidal principle, which, on treatment with alkalis, yielded a crystalline base, $C_{12}H_{16}N_2$ (m. p. 116°).

II. *Constituents of the Leaves and Stems.*—An alcoholic extract of this material, when submitted to distillation with steam, yielded a very small amount of an essential oil. The portion of the extract which was soluble in water contained, besides tannin and colouring

matter, a sugar yielding *d*-phenylglucosazone (m. p. 205°), and a considerable quantity of potassium nitrate.

The portion of the extract which was insoluble in water consisted chiefly of resinous material, and was obtained in the form of a dark green powder. This resin was found to contain a number of substances which had also been isolated from the root of the plant, such as hentriacontane, a phytosterol, $C_{27}H_{46}O$ (m. p. 133°), a mixture of fatty acids, and ipuranol. In addition to these, however, it yielded the following compounds: a new monohydric alcohol, *somnirol*, $C_{32}H_{43}O_6 \cdot OH$, decomposing at 205° and having $[\alpha]_D + 34.8^\circ$; a new dihydric alcohol, *somnitol*, $C_{33}H_{44}O_5(OH)_2$, decomposing at about 250°, and having $[\alpha]_D + 21.2^\circ$; and an acidic, hydrolytic product, *withanic acid*, $C_{29}H_{45}O_6 \cdot CO_2H$ (m. p. 226°), the *methyl* ester of which decomposed at 255°.

Inasmuch as the *Withania somnifera*, unlike some other solanaceous plants, had been found to contain no mydriatic alkaloid, it was deemed of interest to ascertain whether the sedative or hypnotic properties attributed to it could be confirmed. For this purpose, some tests were kindly conducted for us at the Wellcome Physiological Research Laboratories by Drs. H. H. Dale and P. P. Laidlaw, to whom our thanks are due. It was thus ascertained that alcoholic extracts, representing about 7 grams of the root and 3 grams of the leaves and stems respectively, when administered to a dog had no perceptible effect. The hypodermic injection of the alkaloidal principle obtained from the root likewise produced in a dog no symptom of narcosis or other definite result.

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